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Analytical Methods for Oxidative Potential in Trace Elements Bound to Particulates Samples Collected from Urban Locations and Human Health Effects

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Abstract: Analytical methods measuring oxidative potential (OP) are crucial when evaluating ambient particulate matter (PMs) for toxicity. The respiratory tract lining fluid assay (RTLF), Ascorbate assay, chemiluminescent reductive Acridinium triggering (CRAT), dithiothreitol assay (DTT), EPR/ESR (electron paramagnetic/spin resonance) and the dichlorofluorescin assay (DCFH) were reviewed in this study. PubMed and Web of science databases are assessed, susceptibility of each the methods to various PM species composition, PM size distribution, and a comparison is made of seasonality. The DTT assay, in contrast, is typically the recommended technique since it offers a more thorough measurement utilizing quinones and transition metals accumulated in the fine particulates. The OPDTT is found to be contributed by specific transition metals (e.g., Mn, Cu, Fe) and quinones. Inversely, while PMs species' redox characteristics. Epidemiological studies pertaining to oxidative potential caused by PM exposure benefit from the selection of suitable OP measurement techniques and the precise analysis of the relationship between the techniques and PM components. This search produced 402 records on the two databases. Having remove the duplicates a total of 318 records were screened by title and abstracts for inclusion. A total of 50 full text articles were used in this review.

Keywords: Analytical Methods, Trace Element Oxidative Potential, Particulate Matter

ABBREVIATIONS

AA – Ascorbic acid
PM- Particulate matter
OP– Oxidative potential
DTT– dithiothreitol
HPLC– High liquid performance liquid chromatography
EPR- Electron paramagnetic resonance
NPQ– 1, 2 –naphhoquinone
ROS- Reactive Oxygen Species
ESR- Electron Spin Resonance
CRAFT- Chemiluminescence Reductive acridiniumtriggering
GSH- Glutathione
RAM- Redox actve metals
RTLF- Respiratory Tract Lining Fluid
EDTA- Ethylene Diamine Tetraacetic Acid
DTNB-5, 5-dithiobis- (2-nitrobenzoic acid

INTRODUCTION

Facts abound that there is a relationship between trace metals bound to Particulate Matter (PM) toxicity and its health effects (Bollati et al.,2010). There is a significant chance that particulate matter will bind to harmful metals, which could subsequently enter the body by inhalation and have physiological repercussions. The ability of metals to create reactive oxygen species (ROS) in living things, which results in oxidative stress, is the reason behind their toxicity. Research has demonstrated a connection between oxidative stress and the harmful consequences of air pollution on human health. (Bates et al.,2019; Li et al.,2013; Weichenthal et al.,2019). As a result, to further explain air pollution,

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the oxidative potential of airborne particles has been determined and added to PM mass (Weichenthal et al.,2019, Brook et al.,2010 Shi et al.,2003, Zielinski et al.,1999, Kelly 2012).

Assessing the human health risk connected with trace metals bound to PM, oxidative potential has been used as an indicator to measure their health effects. One potential physiological pathway is the capacity of airborne particles to produce reactive oxygen species (ROS), which can cause oxidative stress. However, there is a dearth of toxicological research on oxidative potential of redox active metals and airborne particle databases, along with ROS. (Donaldson et al., 2003, Nel, 2003).

Oxidative potential (OP) is the ability of particulate matter to form reactive oxygen species (ROS) through the creation of oxidants or the consumption of antioxidants, such as hydroxyl radicals (•OH), superoxide (O^{2-}), alkoxyl radicals (RO), peroxyl radicals (RO_2), and so forth. (Aileen and Aleksanta 2013).

As a result of the reaction between the redox-active components of PM, such as quinones and transition metals, and the antioxidants in the respiratory organ lining fluid (ROLF), oxidation products are formed and antioxidants are depleted. There are two ways by which PMs generate ROS in the human body:

(1) Oxidants on or in the Particle itself that deposit in the Respiratory Systems

(2) Certain compounds in PMs stimulate cells to produce extra ROS, or a specific biochemical interacts with PM components to produce ROS as determined by the Fenton reaction, which produces more hazardous hydroxyl radicals from hydrogen peroxide (Li et al.,2013, Stefan and Liochev 2013).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^- \qquad \text{Equation (1)}$$
$$\bullet OH + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \qquad \text{Equation (2)}$$
$$Fe^{3+} + OH \rightarrow Fe^{2+} + OH^- \qquad \text{Equation (3)}$$
$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^+ \qquad \text{Equation (4)}$$
$$\bullet OH + \bullet OH \rightarrow H_2O_2 \qquad \text{Equation (5)}$$

Metal-induced production of free radicals in the body can change calcium and sulfhydryl balance, modify DNA, and increase lipid peroxidation Valko 2005. Water-soluble transition metals have also been found to have a significant role in the generation of reactive oxygen species (ROS) using various ROS probes, including the DTT (dithiothreitol) (Charier and Anestasio 2012), AA (ascorbate acid) (Fang et al.,2015), and macrophage assays (Saffari et al., 2014a, Verma et al.,2015). The highly reactive nature of ROS is because of the unpaired electrons in them.

According to (Perrone et al.,2016 & Simonetti et al.,2018), acellular assays are more advantageous than cellular procedures in terms of reading speed, cost, control settings, and automation compatibility. Because of the limited specificity of the acellular OP test for ROS or specific types of ROS-inducers, no standard technique has been developed to evaluate the toxicity of ambient particles. Because the acellular methods are particular to ROS, none of them have been applied as a standard technique to evaluate the toxicity of ambient particles.

For this purpose, several methods that are commonly used were described and this systemic review described the different analytical techniques documented in published works to determine the oxidative potential of redox active metals bound to particulate matter RAM and provides a summary of methods used to determine metal sources.

This study aim is to examine the body of knowledge about the oxidative potential, sensitivity, and health risk assessment of trace metals attached to airborne particulate matter in analytical procedures.

METHODOLOGY

Search Method. A literature search was carried out without any limitations when utilizing phrases on the databases of Google Scholar, PubMed, and Web of Science, such as oxidation potential, reactive oxygen species, test types in all studies, and trace metals attached to particulate matter.

Inclusion Criteria: For a publication to be included, the data must focus on oxidation potential of metals bound to airborne particulates or on the analytical methods to determine oxidation potential.

Exclusion Criteria**:** Publications that do not report oxidation potential of metals bound to airborne particulates or on the analytical methods to determine oxidation potential were excluded.

Study Selection and Data Extraction: Reviewers (EGO and AO) jointly checked the title and abstract of each manuscript to evaluate its eligibility.

Table 1. A PRISMA diagram providing an overview of the literature selection procedure.

Identification	Records identified through database searching (n= 402) {PubMed (n=110) web of Science (n= 292)	Additional records retrieved by hand-searching the references (n=4)
Screening	Records after duplicates removed (n=318)	Records excluded at title/abstract (n=290)
Eligibility	Full-text articles reviewed (n=28)	Full-text articles excluded with reasons n=(6)
Inclusion	Studies included (n=22)	

RESULTS

Included Studies: This search produced 402 records on the two databases. Having removed the duplicates a total of 318 records were screened by title and abstracts for inclusion. A total of 282 records were removed at this stage, 28 full text articles were reviewed, also additional 22 full text articles were later included in this review.

The PRISMA diagram showing the summary of the selection steps is given in Table 1above. This result focus on the summary of the analytical methods that were used for determination of oxidative potential of redox active metals in airborne particulate matter, sensitivity and their health risk.

Quantifying the Oxidative Potential

Particulate matter toxicity has been shown to be significantly correlated with oxidative potential. A few acellular techniques are employed to measure the OP and illustrate the many processes involved in the production of ROS (Bates et al.,2019). Some methods (e.g., RTLF assay, AA assay) quantify the OP of PMs by measuring the loss of an endogenous antioxidant species or a surrogate of cellular reductants (e.g., OPDTT). The ESR assay quantifies the production of hydroxyl radicals when H_2O_2 is present. Both the emitted light intensity in the CRAT assay and the fluorescence intensity in the DCFH assay are translated into equal H_2O_2 concentrations.

The choice of inert carbon black as a negative control and residual oil by ash (ROFA) as a positive control was made with the goal of standardizing interlaboratory measurements. Additionally, blank filters are routinely removed and passed through the assay system. (Bates et al.,2019, Aileen and Aleksanta 2013).

Respiratory Tract Lining Fluid Assay

It has been demonstrated that the respiratory tract lining fluid (RTLF), which serves as the first detoxifying environment that stimulated particulate matter encounters, contains high concentrations of reduced glutathione (GSH), ascorbic acid (AA), and uric acid (UA) in addition to small molecular weight antioxidants (Zielinski *et al* .1999) Figure 1 below shows the structure of urate and the results of its first degradation. ThiylGS•) radicals are frequently produced by the tripeptide GSH when it reacts with reactive species found in PMs. Equation (3) states that any available GS− can react fast with GS to create glutathione radical disulfide anion ($GSSG^{\bullet-}$) and then $O^{2\bullet-}$. This technique, which involves mixing with a particle suspension, measures variations in the concentration of antioxidant molecules in the liquid that lines the simplified artificial human respiratory tract (Crobeddu et al.,2017).

The pH of the PMs sample and the 200 µM composite antioxidant solution was adjusted to 7.0. After that, the mixture was placed in an incubator that was kept at 37°C for four hours. Following incubations, antioxidant concentrations were assessed using glutathione-reductase enzyme recycling and high-pressure liquid chromatography (HPLC), respectively (Gao et al., 2020). In addition to revealing the quantitative oxidative potential of PMs, the degree to which antioxidants were depleted by PMs also revealed probable physiological effects at the air-lung interface.

$GSH \Leftrightarrow H+ + GS-$ Equation (6)

$GS\bullet + GS- = GSSG\bullet-$ Equation (7)

$O_2 + GSSG\bullet- = GSSH + O^{2\bullet-}$ Equation (8)

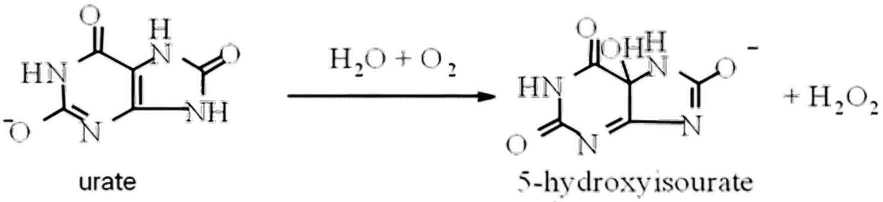

Figure 1. Urate can be oxidized by urate oxidase and forms 5-hydroxyisourate(HIU) initially

The Ascorbate Depletion Assay

The ascorbate (AA) depletion method, which is believed to be a condensed form of the RTLF test, only uses ascorbate acid (Lanfang et al., 2020). As redox active chemicals in PMs are reduced, AA is oxidised to dehydroascorbic acid. Ascorbate's structure and the byproducts of its oxidation and breakdown are depicted in Figure 2. Ascorbate acid is added to the extracts obtained from the particulate matter after incubating it a temperature of 37◦C in this assay. Following an instantaneous response, the reaction was quantified in a UV/VIS spectrophotometer using spectral scans (190–350 nm) spaced ten minutes apart. The PM oxidative potential was represented by the rate at which AA was depleted.

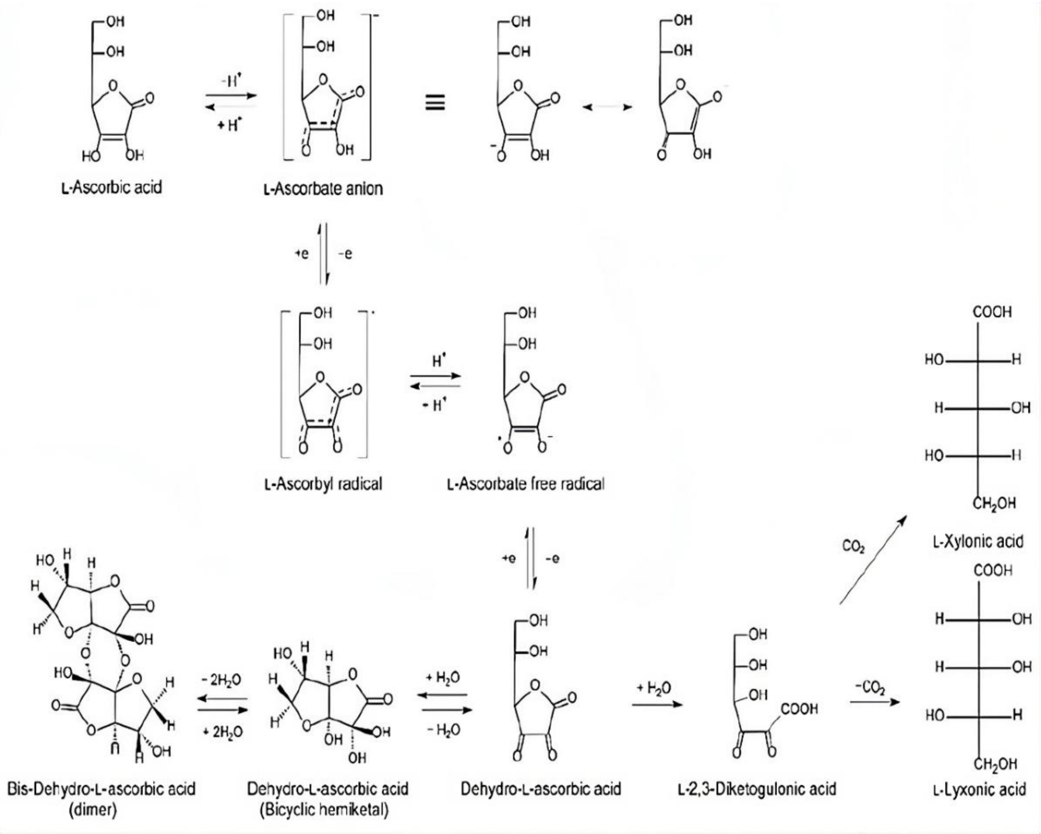

Figure 2. Structure illustrating ascorbate and the products of its oxidation and degradation (Bradshaw *et al*., 2011).

Dithiothreitol Assay

DTT has the ability to reduce oxygen to the superoxide anion ($O_2^{\bullet-}$), which can subsequently create additional reactive oxygen species (i.e., OH). Figure 3 illustrates how redox active chemicals oxidise DTT to its disulfide form and transfer an electron to O_2, creating $O_2^{\bullet-}$. Particles were initially extracted using Milli-Q or methanol in the DTT experiment. Following the extraction process, DTT in phosphate buffer was added and allowed to incubate for a while. An aliquot taken out of the mixture was added to a solution containing 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), trichloroacetic acid (TCA) (quench reaction), and ethylene diamine tetraacetic acid (EDTA) at predetermined intervals. Particles were initially extracted using Milli-Q or methanol in the DTT experiment.

2-nitro-5-thiobenzoic acid (TNB) was produced by the reaction of leftover DTT and DTNB, and its concentration could be determined at 412 nm using a UV/VIS spectrophotometer. When DTT was present in abundance, the concentration of redox-active species in the PMs Sample was directly correlated with the consumption rate of DTT, which depended on the linear slope of DTT depletion. The OP responses might be normalized using the mass of the PMs to indicate the PMs' inherent capacity

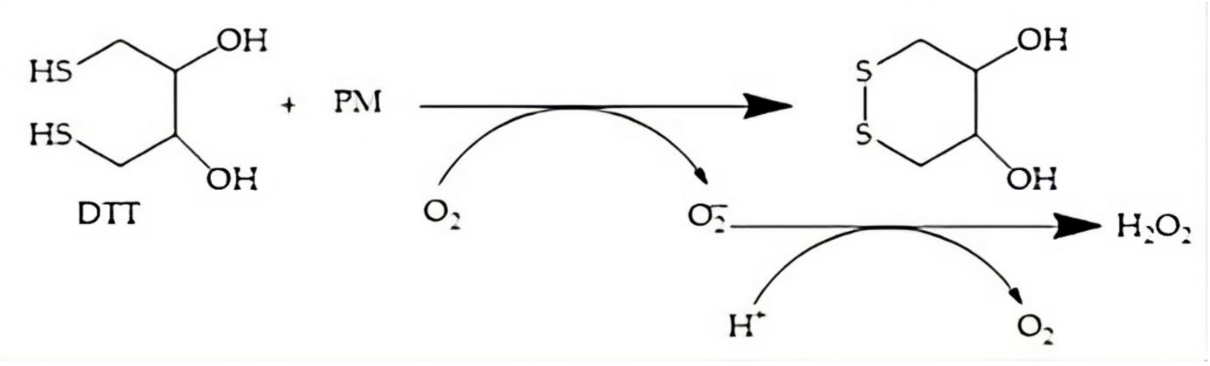

Figure 3. Using particulate matter (PMs) as a catalyst, dithiothreitol (DTT) O2 reaction (Xiong *et al*.,2017)

Chemiluminescent Reductive Acridinium Triggering Assay

This process involves the chemiluminescence of acridine esters in an alkaline environment. Figure 4 illustrates the reaction's mechanism. In the CRAT test, reductants such as DTT or GSH were used to create H_2O_2, which interacted with acridinium ester when a slightly alkaline buffer was added to the solution. Using a luminescence metre, the amount of light released during this reaction was measured in roughly 1 second. One may use the light's intensity to measure the rates at which H_2O_2 is produced. The CRAT approach is still relatively new and hasn't been applied extensively in extensive studies on air pollution.

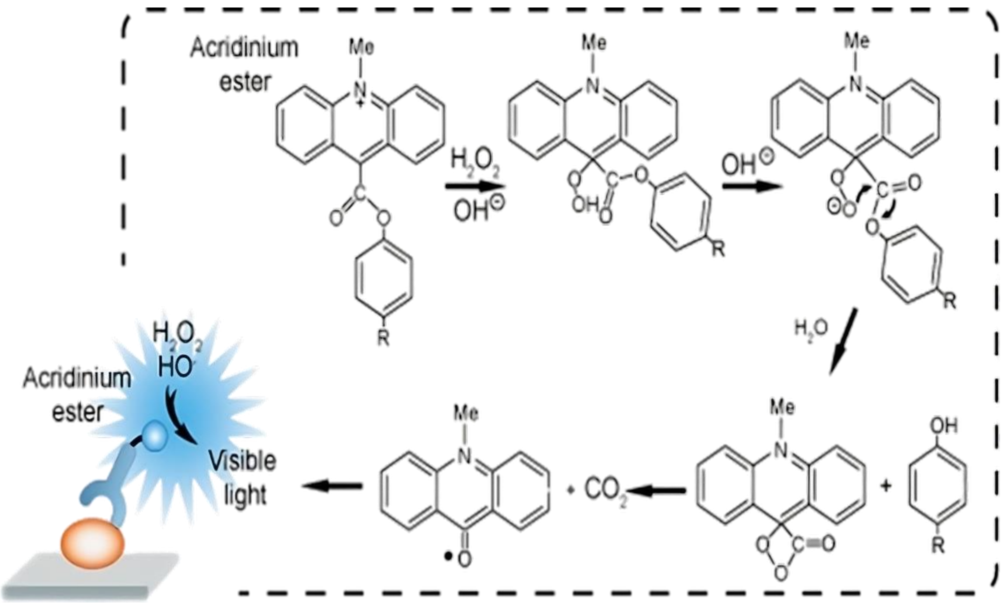

Figure 4. Chemiluminescence reaction mechanism of Chemiluminescent Reductive acridiniumtriggering (CRAT) assay (Zomer et al., 2011)

Dichlorofluorescin Assay

Based on the principle that dichlorofluorescin reagent fluoresces when oxidised, this approach is developed. DCFH quickly oxidised to a fluorescent compound (DCF) in the presence of horseradish peroxidase (HRP) (Figure 5). Before analysis, horseradish peroxidase (HRP) and DCFH in sodium phosphate buffer (pH = 7.2) were combined in a ratio. This experiment lowers the variability of the background H_2O_2 content and was conducted in the dark. To remove ROS from the particles, sample filters were suspended in a beaker containing DCFH–HRP and sonicated. Using fluorescence at 485 nm for excitation and 530 nm for emission, the DCF that was produced was measured. The H_2O_2 calibration curve was utilised in conjunction with least-squares analysis to transform the measured fluorescence intensities into equivalent amounts of H_2O_2 Venkatachari *et. al*., 2005

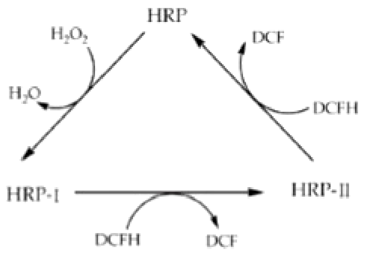

Figure5. H_2O_2andhorseradish peroxidase (HRP) reaction, follow by the reaction with dichlorofluorescin (DCFH) to produce fluorescence product fluorescent compound (DCF)
(Fuller et.al., 2014)

The spin resonance assay and paramagnetic electron assay

This technique was created for the direct identification and measurement of substances such free radicals and transition metal ions that contain unpaired electrons. Free radicals from particles gathered on filters can be used to measure the physical assay. Increased free radical signals produced by burning coal at a finer temperature.

This technique was created for the direct identification and measurement of substances such free radicals and transition metal ions that contain unpaired electrons. Free radicals from particles gathered on filters can be used to measure the physical assay. It is possible to measure stronger free radical signals produced by coal combustion fine particulates (PM< 1.1𝓜m).

The free radical spectrum pattern varied with different spin traps, and the ESR assay can also be used to analyse different free radicals with different spin traps. Four peaks were detected in the 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) spin trap, however only three peaks were detected in the 1-hydroxyl-2,2,6,6-tetramethyl-4-oxo-piperidine (TEMPONE-H) spin trap.

Particles' oxidative potential on filters is determined by their ability to generate hydroxyl radicals through a Fenton-type reaction when hydrogen peroxide is present and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) is used as a specific spin-trap. Shi *et al.*, 2003.

Apart from DMPO, an additional spin-trap 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine (TEMPO-H) was modified to measure the overall concentrations of reactive oxygen species (ROS) (Figure 7). When TEMPONE-H reacts with radicals, it can deprotonate, converting it from a closed shell molecule to a radical. Equation (6) states that TEMPONE-H decreases peroxy nitrite to NO_2, and Equation (7) states that it reduces NO_2 to nitrite. Additionally, when TEMPONE-H reacts with peroxy and superoxide radicals (Equation (9) and (8), 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidinoxyl TEMPONE is formed. Tong *et al* 2018, Dikalov *et al.*, 1997

$C_6H_{11}NO + \bullet OH = C_6H_{11}NO\text{-}OH$ Equation (9)
$C_6H_{11}NO + O_2^{\bullet -} \rightarrow C_6H_{11}NO\text{-}OOH \rightarrow C_6H_{11}NO_2\text{-}OH$ Equation (10)
$C_9H_{17}NO_2.HCl + ONOOH \rightarrow C_9H_{16}NO_2 + NO_2 + HCl + H_2O$ Equation (11)
$C_9H_{17}NO_2.HCl + NO_2 \rightarrow C_9H_{16}NO_2 + HCl + NO_2$ Equation (12)
$C_9H_{17}NO_2.HCl + H_2O \rightarrow C_9H_{16}NO_2\ H_2O_2 + HCl$ Equation (13)
$C_9H_{17}NO_2.HCl + RO_2 \rightarrow C_9H_{16}NO_2 + RO_2H + Cl$ Equation (14)

Table 2. Provides an overview of the features of the analytical methods that were assessed for oxidation potential determination and risk evaluations for human health.

Method	Experimental details	Samples	Year	Reference
DTT	the rate at which chemical proxies for cellular reductants reduce	$PM_{2.5}$	2017, 1997 2018	Lyu*etal.*,2018, Janssen*etal.*,2014
AA	the antioxidant loss rate	PM_{10}, $PM_{2.5}$	2018, 2019	Perrone *et al.*,2016 Simonetti *et al.*,2018
CRAFT	the chemiluminescence reaction	Ambient particles	2014	Yang *et al*.,2014
DCFH	the gradual increase in fluorescence intensity	anthropic combustion; secondary aerosol	2018	Simonetti *et al*.,2018
ESR	PMs' capacity to generate •OH	ambient particles	2014	Janssen *et al.*,2014
GSH	the rate of antioxidant loss	emissions from non-exhaust traffic	2019	Weichenthal *et al.*2019

Table 3. Evaluation of the oxidative potential through comparison of acellular assays. Adapted from (Bollati *et al.*,2010)

Method	**Advantages**	**Estimated Method**	**Sensitivity**	**Sources**	**Quality**
DTT	rapid, cheap, Simple to carry out and appropriate for automation	the rate at which chemical proxies for cellular reductants are depleted	Inorganic ions, metals connected to traffic, and organic compounds	burning of biomass; braking and tyre wear; combustion of fossil fuels in traffic; and photochemical ageing	connected to the fine fraction
AA	fast, inexpensive, Simple to execute and ideal for automation	antioxidant degradation rate	Metals	non-exhaust traffic emissions	related with rough particles
GSH	fast, inexpensive, easy to perform and suitable for automation	The rate of antioxidant loss	Cu	non-exhaust traffic emissions	Ineffective as a traffic marker
DCFH	quick, affordable, simple to execute, and automatable	the increase in fluorescence intensity over time	inorganic ions, Organic compound	combustion by humans; secondary aerosol	linked to the fine fraction Highly correlated with PM mass concentration connected to both fine and coarse particles
CRAT	not been utilised extensively	chemiluminescence process	transition metals, quinones	ambient particles	
ESR	comparatively little and inexpensive	PMs' capacity to produce •OH	transition metals , organic components	surroundings particles	

DISCUSSION AND CONCLUSION

Over time, analytical techniques have been developed to determine the oxidation potential of trace elements that are bound to particulate samples. The following techniques are used for the OP of RAMs bound to airborne particulate matter: RTLF, AA, GSH, DTT, CRAT, DCFH, and ESR. After comparing the sensitivity to various PM species compositions, it was determined that the DTT assay might be the best approach because of its sensitivity to tracers of redox active metals and aromatic organic compounds derived from combustion. Quinones and particular redox active metals (such as Mn, Cu, and Fe) significantly influenced the OPDTT value. When assessing the oxidative potential of PMs, it is imperative to consider the insoluble fraction of PMs as well as the interactions among PMs Components that may alter the redox properties of PMs species. A combination of two or more OP measures may also be required to create an accurate predictor for health effects, given that the OP has multiple dimensions.

Understanding the oxidative potential of trace elements bound to particulate matter is crucial for toxicological studies and public health. Recommendations for mitigating the health risks associated with urban air pollution include:

- Stricter regulations on industrial emissions
- Promotion of cleaner transportation alternatives
- Development of effective air pollution control technologies
- Public awareness campaigns to promote healthy lifestyle choices

By implementing these measures, policymakers can significantly reduce exposure to oxidatively potent trace elements and protect public health. Collaborative efforts between researchers, policymakers, and the public are essential for addressing the challenges posed by urban air pollution and its impact on human health.

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